

REMARKS/ARGUMENTS

Claims 1-20 remain in this application. Applicants have amended the specification and figures to insert the sequence listing information. Accordingly, no issues of new matter are believed to be raised by the above amendments. Applicants will submit formal drawings reflecting the above amendments to Figs. 1, 2, 5, and 8 upon approval by the Examiner.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page(s) is/are captioned "Version with markings to show changes made".

Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

By: 

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please insert the attached sequence listing before the claims which start on page 38.

Please replace the paragraph beginning on page 13, line 10 with the following rewritten paragraph:

--To generate p(-3348/-150)SCD-luc (c1), clone 19 was digested with *Pst*I and *Sac*II and blunted with T4 polymerase. All sequences herein are numbered relative to the translation start site, designated +1. The 3.2 kb fragment containing only the 5' end of the gene was cloned into a blunted *Hind*III site of the luciferase vector pGL3-basic (Promega, Madison, WI, USA). p(-2328/-150)SCD-luc (c2) and p(-1295/-150)SCD-luc (c4) were generated by digesting the clone 19 with *Taq*I/*Sac*II and *Kpn*I/*Sac*II, respectively, and the resulting fragments were subcloned into a blunted *Hind*III site of pGL3-basic. p(-1537/-150)SCD-luc (c3) and p(-882/-150)SCD-luc (c5) were subsequently generated by digesting c1 with *Sac*I and *Sma*I, respectively. The resulting fragments were religated using T4 ligase. p(-461/-150)SCD-luc (c6) was generated by digesting c4 with *Eae*I/*Nco*I and the resulting fragment was ligated to blunted *Nco*I/*Hind*III sites of pGL3-basic. p(-270/-150)SCD-luc (c7) was created by digesting c4 with *Hinc*II and *Nco*I. The resulting fragment was subcloned into *Nco*I/*Hind*III digested, blunted, pGL-3 basic. p(-753/-150)SCD-luc (c8), p(-609/-150)SCD-luc (c9) and p(-496/-150)SCD-luc (c10) constructs were assembled by polymerase chain reaction using one of the three specific sense oligonucleotides (bp -753 to -732, c8), 5'-GGTTCACCACTGTTTCCTGAGA-3' (SEQ ID NO: 1); (-609 to -688, c9) 5'-GATGCCGGGCAGAGGCCAGCG-3' (SEQ ID NO: 2); (-496 to -474, c10), 5'-GGCAACGGCAGGACGAGGTGGCA-3' (SEQ ID NO: 3); and a common antisense oligonucleotide (-166 to -145) 5'-CCGCGGTGCGTGGAGGTCCCCG-3' (SEQ ID NO: 4). All PCR reactions were conducted with proofreading Turbo *PFU* DNA polymerase (Stratagene, La Jolla, CA, USA), and the original c5 construct was used as template. Amplification products were phosphorylated with T4 kinase and subcloned into the *Hind*III blunted site of pGL-3 basic. The sequence of the PCR products were examined for accuracy using the dideoxynucleotide chain termination method.--

Please replace the paragraph beginning on page 14, line 8 with the following rewritten paragraph:

-- Total RNA was extracted from human hair follicle biopsies and cultured HaCaT keratinocytes (Boukamp *et al.*, 1988) using RNazol (TEL-TEST Inc., Friendswood, TX, USA). A *SCD* fragment was amplified with PCR, using clone 19 as a template with the following primers: sense (-275 to -256), 5'GCCAGTCAACTCCTCGCACT3' (SEQ ID NO: 5); antisense (+7 to +27), 5'ATCGTCCTGCAGCAAGTGGGC3' (SEQ ID NO: 6). This resulted in a 302 bp fragment (nucleotide -275 to +27) which was subcloned into the TA cloning vector pCR2.1 (Invitrogen, Carlsbad, CA, USA). The plasmid (NPA1-pCR2.1) was further digested by XbaI and EcoRV to release unnecessary vector sequence between the insert and the T7 promoter and religated with T4 ligase. The final construct (NPA2-pCR2.1) was linearized by HindIII and a labeled antisense RNA (400 bp) was synthesized from the T7 promoter of the plasmid using T7 RNA polymerase (Ambion, Austin, TX, USA). Following synthesis, the full-length probe was gel purified. Total RNA (15ug per sample) from human hair follicles and HaCaT keratinocytes was hybridized to the labeled transcript for 16h at 42°C. The RNA samples were digested with an RNase mixture (Ambion, Austin, TX, USA), and the sizes of the protected RNA were determined by electrophoresis on a 5% denaturing polyacrylamide gel, using a DNA sequencing ladder generated from NPA2-pCR2.1.--

Please replace the paragraph beginning on page 14, line 29, with the following rewritten paragraph:

--Nuclear extracts of HaCaT keratinocytes were prepared using standard procedures (Dignam *et al.*, 1983). Protein concentrations of the extract were determined with BCA assay (Pierce, Rockford, IL, USA). Electrophoretic mobility shift assays were performed with a Gel Shift assay system according to the manufacture's instruction (Promega, Madison, WI, USA). Briefly, hybridization was performed at room temperature for 20 min in a volume of 20 ul consisting of 0.1 pmol of probe (70, 000 cpm) and 5 ug of nuclear extract. The gel shift mixture was incubated for 20 min at room temperature, and samples were immediately electrophoresed at 150V for 3 h on a 5% nondenaturing acrylamide gel using 0.5 x TBE running buffer (34 mM Tris.borate, 0.75 mM EDTA) prerun at 100 V for 100 min. The gel was then dried for autoradiography. For competition studies, radioinert DNA competitor was added as a 100-fold molar excess and preincubated with the nuclear extract at room

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temperature for 5 –10 min. The ³²P-labeled DNA was then added to the mixture and incubated at room temperature. The oligonucleotides used for EMSAs were as follows: AP-2 (sense, -595 to -575), 5'-GCCCAGCGGCGGGTGAAGAG-3' (SEQ ID NO: 7); AP-2 (antisense), 5'-CTCTTCCACCCGCCGCTGGGC-3' (SEQ ID NO: 8); SP-1 (sense, -559 to -539), 5'- AACAGAGGGGAGGGGGAGCGA-3' (SEQ ID NO: 9); SP-1(antisense), 5'-TCGCTCCCCCTCCCCTCTGTT-3' (SEQ ID NO: 10); CCAAT (sense, -509 to -484), 5'GCGCCGAGCCAATGGCAACGG-3' (SEQ ID NO: 11); CAAT (antisense), 5'-CCGTTGCCATTGGCTCGGCGC-3' (SEQ ID NO: 12).--

Please replace the paragraph beginning on page 16, line 8 with the following paragraph:

--Mutation in the binding sites for transcription factors SP-1 and CCAAT were introduced into the plasmid *c9*, containing the 0.5 kb *SCD* promoter subcloned into the blunted HindIII site of pGL-3 basic, using the Quick Change Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA, USA). Two double-stranded oligonucleotide primers containing the desired mutations are as follows (underlined base pairs denote mutant substitutions): 5'-AAGGAGAAACAGAGGAAAAGGGGGAGCGAGGAGCTG-3' (SP-1) (SEQ ID NO: 13), and 5'-AGCAGATTGCGCCGAGAAAAATGGCAACGGCAGGAC-3'(CCAAT) (SEQ ID NO: 14).--

Please amend Figures 1 (page 4), 2, 5, and 8 as shown in the attached informal drawings.